Neuroprotective and neurodegenerative effects of the chronic expression of tumor necrosis factor α in the nigrostriatal dopaminergic circuit of adult mice

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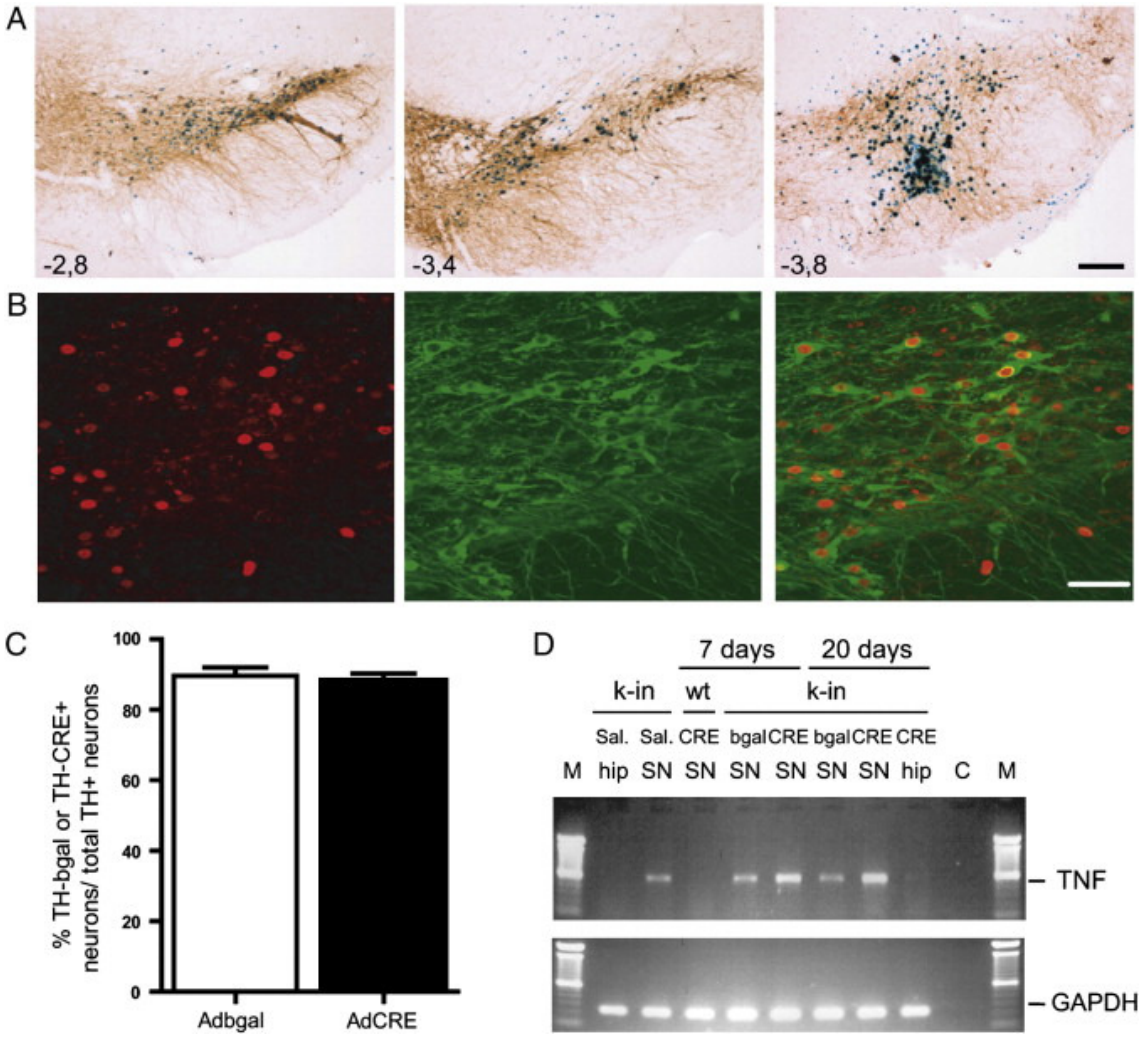
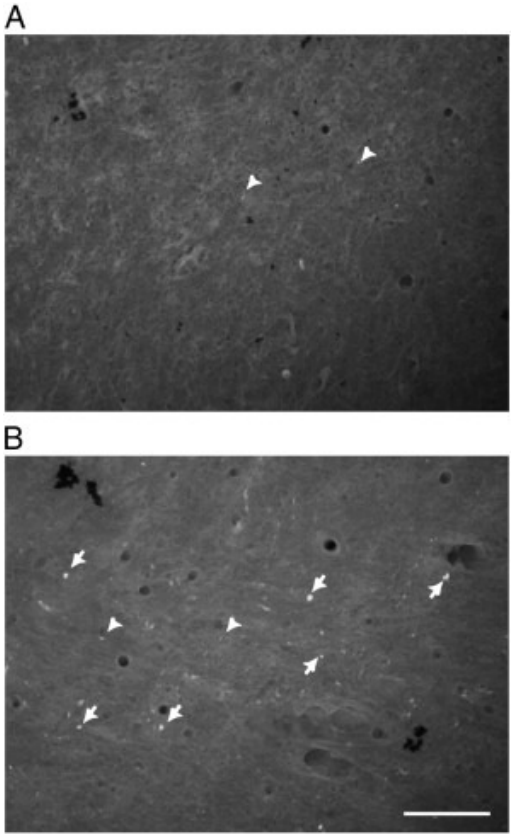
Fluoro-jade staining

30 μm coronal sections at the level of the SN were mounted onto Superfrost Plus slides and dried at room temperature for 48 hours. Slides were then immersed in a solution containing 1% NaOH in ethanol 80% (5 min), then in 70% ethanol (2 min) and finally in distilled water (2 min). Slides were transferred to a freshly prepared solution of 0.06% potassiun permanganate and gently shaken for 10 min. After a rinse in distilled water for 2 min, slide-mounted sections were placed in 0.0004% Fluoro-jade B dissolved in 0.1% acetic acid in the dark for 20 min, and gently shaked. After staining, sections were washed three times in distilled water (1 min each wash), dried, immersed for 1 min in xilene and coverslipped.



Supplementary Fig. 1. Characterization of the system combining K-in mice, adenoviral vectors and the CRE/loxP system to achieve different levels of TNF-α expression in the SN. A) X-gal staining and immunohistochemistry for tyrosine hydroxylase (TH) of nigral sections injected with Adbgal. Three representative sections, at positions AP-2.8, -3.4, and -3.8 from the *bregma* according to Watson and Paxinos and encompassing the entire SN, are shown. B) Detection of nuclear CRE (left panel) and TH (center panel) and co-localization of CRE and TH (right panel) in the SN as analyzed by double immunofluoresence. C) Quantification of double-labeled TH/beta-galactosidase or TH/CRE cells throughout the entire SN relative to total TH+ cells 4 days after adenoviral transduction in that region. D) RT-PCR of the SN or hippocampus of K-in (K-in) mice or control littermates (wt) injected with adenovectors or saline in the SN. Amplification of GAPDH RNA retrotranscribed and amplified as the rest of the samples was used as a control of the amount of RNA subjected to PCR. Hip, hippocampus. Scale bar in A: 200 μm; in B: 20 μm.



Supplementary Fig. 2. Neurodegeneration after 100 days of AdCRE treatment. A–B) Representative SN sections stained with Fluorojade-B 100 days after Adbgal (A) or AdCRE (B) injection. Arrow: degenerating neuron; arrowheads: red blood cell. Scale bar: 50 μm.